

Ecological issues related to ozone: agricultural issues

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Abstract

Research on the effects of ozone on agricultural crops and agro-ecosystems is needed for the development of regional emission reduction strategies, to underpin practical recommendations aiming to increase the sustainability of agricultural land management in a changing environment, and to secure food supply in regions with rapidly growing populations. Major limitations in current knowledge exist in several areas: (1) Modelling of ozone transfer and specifically stomatal ozone uptake under variable environmental conditions, using robust and well-validated dynamic models that can be linked to large-scale photochemical models lack coverage. (2) Processes involved in the initial reactions of ozone with extracellular and cellular components after entry through the stomata, and identification of key chemical species and their role in detoxification require additional study. (3) Scaling the effects from the level of individual cells to the whole-plant requires, for instance, a better understanding of the effects of ozone on carbon transport within the plant. (4) Implications of long-term ozone effects on community and whole-ecosystem level processes, with an emphasis on crop quality, element cycling and carbon sequestration, and biodiversity of pastures and rangelands require renewed efforts.

The UNECE Convention on Long Range Trans-boundary Air Pollution shows, for example, that policy decisions may require the use of integrated assessment models. These models depend on quantitative exposure–response information to link quantitative effects at each level of organization to an effective ozone dose (i.e., the balance between the rate of ozone uptake by the foliage and the rate of ozone detoxification). In order to be effective in a policy, or technological context, results from future research must be funnelled into an appropriate knowledge transfer scheme. This requires data synthesis, up-scaling, and spatial aggregation. At the research level, interactions must be considered between the effects of ozone and factors that are either directly manipulated by man through crop management, or indirectly changed. The latter include elevated atmospheric CO₂, particulate matter, other pollutants such as nitrogen oxides, UV-B radiation, climate and associated soil moisture conditions.

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1. Introduction

Over the course of several decades, the research community has addressed the effects of elevated levels of tropospheric ozone on agricultural crops. Findings of negative impacts on crop productivity have raised public concern first in the United States, and later in Europe. More recently, the issue has arisen in other parts of the world, in particular in Southeast Asia. Recent modelling work by the United Kingdom Meteorological Office, using the IPCC-A2 emission scenario, indicates the possible expansion of the area subject to elevated summertime ozone over the next decades with hotspots in North and South America, Europe, and Asia

(Collins et al., 2000) (Fig. 1). Thus, when we look at the future needs for research on ozone effects, we should keep in mind the global dimension of the problem. It must be placed in context with a series of other global change phenomena, as well as with trends in socioeconomic developments in different parts of the world.

At the beginning of the 21st century, we are faced with a rapid change in the environment. Greenhouse forcing is expected to alter temperature and rainfall patterns, and atmospheric CO₂ concentration will continue to increase for an extended period of time. There can be no doubt that under future conditions, agro-ecosystems and their management will substantially differ from today, and potential ozone impacts in the agricultural sector will depend largely on changes in other factors. Hence, the evaluation of future trends in ozone impacts cannot rely solely on trends in ground-level ozone concentrations predicted by atmospheric

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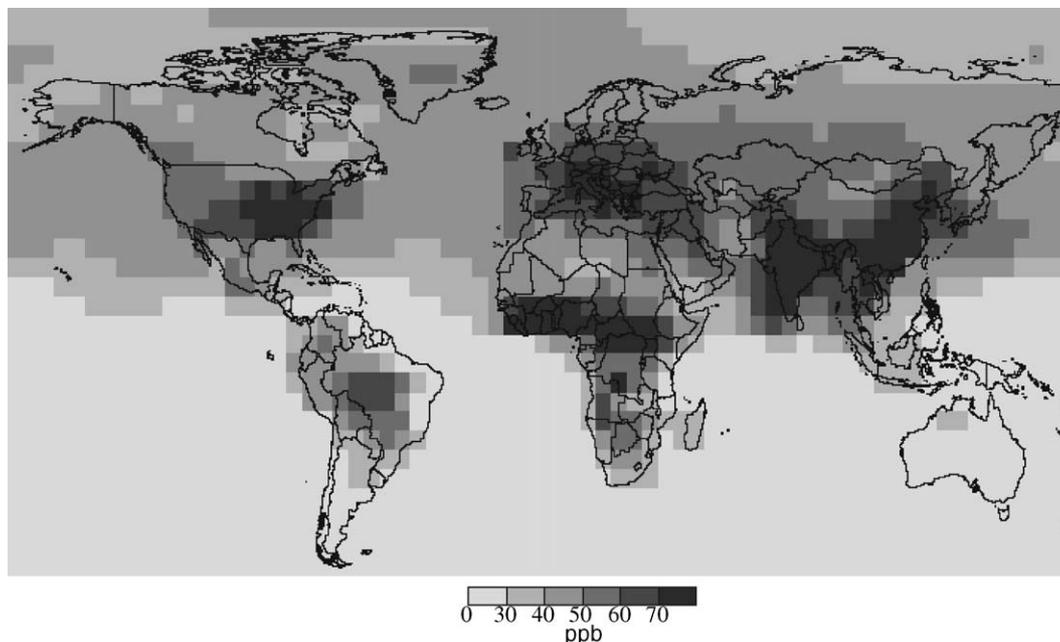


Fig. 1. Mean maximum growing season O_3 concentrations for 2030. Data produced by the STOCHEM global ozone model (Collins et al., 2000). Courtesy of D. Derwent, UK Meteorological Office.

models. Also, research aimed to support the future development of environmental policies should not view ozone pollution in isolation but should consider interactions of ozone with the physical and chemical changes in the environment. At the same time, the potential for natural and man-made adaptation of crops to ozone and other stresses must be considered (Barnes et al., 1990).

In many countries where the demand for food is stabilized or only slowly increasing, the importance of domestic agricultural production and the constraints by the environment may be declining. According to FAO statistics, wheat production in the US has been relatively stable since 1980, and has increased by some 40% in Europe, which is small compared to advances—for instance, in China where it has doubled. Whether or not agricultural production will be able to keep pace with the growing food demand in rapidly expanding populations will depend on many factors, but possibly also on the future levels of air pollution, including tropospheric ozone (Chameides et al., 1999). The risk for negative effects of ozone on crop productivity may create the need for new crop varieties that are better adapted to ozone stress, as in the case of pests and diseases. With an improved understanding of the mechanisms underlying ozone tolerance, biotechnological advances may be sought as the key to secure future food supply in increasingly polluted regions where air pollution abatement is not sufficiently effective.

Because the problem of ozone pollution for agriculture has been recognized in the US and in several European countries for some time, there may be a shift in the focus in these countries from addressing the problem itself to addressing the question of how to deal with it. However, in this process of transforming knowledge concerning the problem into practical implementation plans for problem solving, there will be continuous need for research—both fundamental and applied—and expert knowledge to support the decision-making progress. As an example for the link between science and policy, the UN Economic Commission for Europe (UNECE) has set up a structure in support of the Convention on Long-range Trans-boundary Air Pollution (CLRTAP) to ensure the flow of information from basic research to policy (Fig. 2). The aim is to continuously improve the tools and data to assess the risk from various pollutants to humans and nature over large areas, and to develop pollution abatement strategies that are optimized in terms of their feasibility and effectiveness using integrated assessment models (Amann et al., 1999).

The aim of this paper is to address some of these issues, and their inter-linkages. We will attempt to identify the needs for future research by considering the broader view of global change, and the necessity for an integrated approach. We will relate the key issues to important processes that deserve research attention, and to the main target areas. These are the

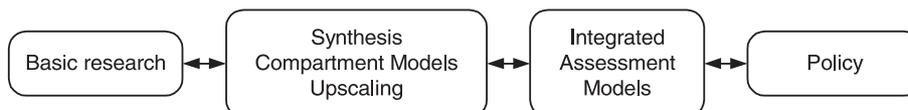


Fig. 2. Linking research to policy in the framework of the UNECE Convention on Long Range Trans-boundary Air Pollution.

development of environmental policies, biotechnological crop improvement, and sustainable agricultural practice.

2. Risk assessments and policy

Air pollution policy aimed at reducing the risk of adverse effects on ecological systems depends on (1) quantitative estimates of the likelihood for adverse effects to occur, and (2) tools to evaluate emission reduction strategies capable of reducing the risk in a cost-effective manner. Specifically, the risk that ground-level ozone poses to agricultural ecosystems, in a particular region or nation, is dependent on the variation of ozone exposure across the area of agricultural land use. The risk is also dependent on the edaphic and climatic conditions prevailing in the region of interest, and the physiological and ecological properties of the exposed systems. Although it has long been recognized that an integral approach to assess the risk would be necessary, it is only recently that the necessary tools are being developed.

2.1. The past

The approach used, to date, was to characterize the ecosystem exposure to ozone using a single statistical index such as a seasonal mean, or cumulative indices with or without some type of concentration-weighting function (e.g., SUM06 in the US, or AOT40 in Europe). This was done to emphasize the importance of cumulative long-term effects (Fuhrer et al., 1997). Based on exposure–response information using this index, air quality standards, guideline values (sensu WHO), or critical levels (sensu UNECE) could be deduced. The area at risk could be estimated based on the difference between such a critical value and the actual measured or modelled exposure. This procedure has been implemented in the RAINS model (Amann et al., 1998) in order to assist the negotiations on the 1999 Gothenburg Protocol to Abate Acidification, Eutrophication and Ground-level Ozone under the UNECE Convention on Long-range Trans-boundary Air Pollution (UNECE, 2001). An integrated assessment tool was used to develop effective European control strategies (Amann et al., 1999). Alternatively, the ozone exposure could be combined with exposure–response functions to estimate the magnitude of the potential ozone impact. With each component of this type of analysis in a separated GIS layer, a spatially explicit risk quantification could be carried out (Hogsett et al., 1997).

This approach has a number of shortcomings. (1) Short-term or long-term dynamics in the emission–exposure–response system are largely ignored. (2) The approach is based on ambient concentrations, regardless of whether ozone is actually absorbed by the vegetation. (3) The concentration at the measuring height is not corrected for systematic effects, such as the concentration profile, in order to justify a link to the effects observed in well-mixed fumigation chambers (Tuovinen, 2000).

2.2. The future

2.2.1. Ozone flux and stomatal uptake

Developments are now in progress that should make it feasible to assess ozone risks dynamically based on measured or modelled ozone flux rather than ozone concentration. This approach assumes that the response of a plant is more directly related to the amount of ozone entering the leaves through the stomata than to the atmospheric concentration. The need to directly assess the damaging component of plant ozone exposure by modelling the stomatal fraction of total ozone deposition has been recognized both in the US (e.g., Panek et al., *in press*) and Europe (Fuhrer, 2000). In fact, considerable progress has been made in the quantification of the ozone flux using models. A detailed discussion of the micrometeorological processes involved, and of the needs for possible improvements of the methodology to infer ozone deposition, is beyond the scope of this paper. However, it should be mentioned that several approaches exist to model the ozone flux to the surface (Soil–Vegetation–Atmosphere–Transfer models, SVAT), as described in detail by Grünhage et al. (2000). These models use either a one-layered resolution of vegetation, or two- or multi-layered resolutions. The latter concept that treats many transfer processes more explicitly, is currently implemented, for instance, in the network (AIRMoN-Dry) of the US National Oceanic and Atmospheric Administration (NOAA) and the US Environmental Protection Agency (EPA) (Meyers et al., 1998). In the UNECE arena, a one-layered resolution is presently used, and ozone flux is modelled as a function of the ozone concentration above the plant canopy and a series of atmospheric, boundary and surface resistances to ozone transfer to the sub-stomatal cavity of an individual leaf (Emberson et al., 2001). Through their effects on the various resistances, a number of environmental factors including atmospheric turbulence, temperature, radiation, soil moisture and atmospheric humidity, as well as specific plant-related factors, such as plant phenology, are considered in the modelling of ozone transport.

Despite the progress made in modelling ozone fluxes, the fate of ozone once it reaches the canopy remains uncertain. In addition to the stomatal flux, the total ozone flux for a specific land cover also includes the parallel flux to external plant surfaces, and the flux to the soil. The partitioning of the total flux into these components, in relationship to the specific surface characteristics, is not fully understood, and the information reported in the literature tends to be inconsistent. Results using the deposition module used in connection with the European Monitoring and Evaluation Programme (EMEP) chemical-transport model suggest that both stomatal and non-stomatal fluxes are comparable in magnitude (Simpson et al., 2001). Because of the interdependence between total flux and stomatal flux, it is important that models calculate total ozone deposition. An additional unknown is the response of the stomatal conductance to absorbed ozone, and, finally, nighttime processes

have been largely ignored in past attempts to characterize critical ozone exposures (Musselman and Minnick, 2000), an issue which needs to be re-examined.

A model for stomatal ozone flux on the pan-European scale was linked to the 3-D EMEP meteorology model that provides values for atmospheric and boundary layer resistances (Emberson et al., 2000). This approach is promising, and improvements in the parameterization of the model are on the way. The aim is to link the model to the EMEP photochemical model for its application in the policy context. The model involves a multiplicative approach using empirical response functions of stomatal conductance to the environmental factors based on Jarvis (1996). In many cases, experimental data underlying these functions are very limited. Of the factors contributing to stomatal conductance, those controlling soil moisture are perhaps the most uncertain (Nussbaum et al., *in press*). Thus, model improvements will crucially depend on new data, and on field validation.

Comparisons between simulations and measurements of ozone fluxes for specific sites with well-defined conditions generally reveal good model performance. For the application of ozone flux models on larger scales, spatially explicit input data are required. However, uncertainty in the input data for environmental conditions, such as soil moisture, wind speed, and leaf area index, may be a major limitation to model performance at the regional or larger scales. Likewise, land cover and land use maps need to be refined. Attempts to reduce the uncertainty in input data needed for scaling-up the models from the local to national or larger scales may benefit from progress made within the climate change research community in the context of modelling impacts on agro-ecosystems.

2.2.2. Risk analysis based on ozone flux

Ozone risk analysis based on ozone flux must consider both the rate of ozone uptake via the stomata and the rate of detoxification. Musselman and Massmann (1999) defined the 'effective flux' (EF) as the balance between uptake into the leaf at a given point in time, $F(t)$, and the defense response, $D(t)$, at that time.

$$EF = F(t) - D(t)$$

EF can be integrated to yield the cumulative effective loading (CEL)

$$CEL = \int_0^T [F(t) - D(t)] dt$$

Using these terms, the overall response of the plant to ozone can then be quantified as the change in a measured plant parameter (e.g., photosynthesis) per CEL. For the application of this approach, it is necessary to scale CEL from individual leaves to the whole canopy, and to relate CEL_{canopy} to system-level plant responses such as yield. Empirical data describing this type of relationship are still

scarce. A few studies have provided some type of empirical relationship between ozone uptake and yield of wheat under open-top chamber conditions (Fuhrer et al., 1992; Gelang et al., 2000), but further work involving experiments specifically designed for this purpose is needed. As an alternative, mechanistic modelling of plant defense reactions may be used. Plöchl et al. (2000) developed a mathematical model (Simulated Ozone Detoxification in the leaf Apoplast (SODA)) that permits, to some extent, the quantification of ozone detoxification, based upon the direct reaction of the pollutant with ascorbate located in the aqueous matrix associated with the cell wall. However, as discussed below, knowledge of reactions involved in the scavenging of ozone in the apoplast is still limited. Based on model simulations and measurements, Zheng et al. (2000) concluded that protection by ascorbate is important but not complete. Hence, model developments must consider other factors acting in combination with ascorbate. But this requires progress in the understanding of the fundamental processes involved in ozone action. Furthermore, defensive mechanisms should be scaled to the canopy level, a scale at which not only biochemical but also physiological and morphological mechanisms are involved, which operate at longer time-scales. For instance, carbon allocation to various plant parts plays a key role, as well as structural adjustments in mixed plant communities.

3. Issues related to ozone action

Carbon assimilation, translocation, nutrient acquisition and other physiological processes are inhibited by ozone exposure that ultimately lead to suppressed plant growth and yield, but the question remains as to how this occurs. The leading hypotheses for these effects and the subsequent decline in plant productivity are listed (Heath and Taylor, 1997; Pell et al., 1997; Sandermann, 1998; Rao and Davis, 2001) as follows: (1) Membrane damage in leaf tissue results in ionic imbalances and other dysfunction. (2) Loss of photosynthetic capacity occurs due to lower levels of Rubisco activity and content. (3) A diminished ability to allocate carbohydrates to developing plant organs. (4) Production of signals leads to pathogen attack or wounding-type responses. (5) Accelerated senescence occurs. A number of recent studies, many of them utilizing molecular techniques, have significantly added to our understanding of how ozone affects plants, but many of the biochemical and molecular mechanisms involved in oxidant injury from ozone remain unclear.

3.1. Processes at the molecular and cellular level

3.1.1. Initial reactions

Reactions of ozone with plants can be classified in three ways (Mudd, 1996): (1) reactions in the solid phase (i.e., with cuticular components of leaves); (2) reactions in the gas phase (i.e., reactions with hydrocarbons emitted by plants);

(3) reactions in the liquid phase that require dissolution of ozone in aqueous media, followed by reaction with lipids, proteins or other cellular components. The most detrimental effects of ozone probably occur after ozone enters the leaf through stomata and involve reactions in the gas and liquid phases. Ethylene emission following exposure to ozone has often been observed. Ozonolysis of alkenes such as ethylene forms aldehydes, hydrogen peroxide and hydroxy-hydroperoxides. It has been proposed that the toxicity of ozone can be attributed in part to the peroxides produced when alkenes react with ozone (Hewitt et al., 1990). However, a resistance model for mass transfer of olefinic compounds from the plasmalemma to the atmosphere indicated that reactions between ethylene and ozone were of minor importance because ethylene concentrations were too low (Chameides, 1989). Ethylene production appeared to be a result of ozone injury rather than a cause (Chameides, 1989), although it may have an important regulatory role in modulating lesion formation and initiating premature senescence (Rao and Davis, 2001; Overmyer et al., 2000).

In the intercellular space, ozone rapidly diffuses into the aqueous media and possibly lipid membrane of the cell where it reacts with cellular components. Ozonolysis is suggested as the primary mechanism of ozone action with lipids, and primarily yields aldehydes and peroxides (Pryor, 1994; Mudd, 1996). In addition, radicals are produced by reactions involving ozone and olefinic compounds such as unsaturated fatty acids and tryptophan or by reactions involving ozone and electron donors such as glutathione, phenolics and ascorbate (Pryor, 1994). One aldehyde, 4-hydroxy-2-nonenal (HNE), is a major metabolite and is the most toxic product of lipid peroxidation that has been identified (Kirichenko et al., 1996). HNE has been shown to be cytotoxic and implicated in numerous forms of cell injury. HNE is highly reactive with biological molecules, preferentially with thiol compounds (glutathione, cysteine) and proteins containing thiol groups. In other studies, however, analysis of the total lipids of ozone-treated RBC ghosts showed no significant change in the distribution of lipid classes, no significant change in the fatty acid composition, and no evidence for the formation of phospholipid ozonolysis product (Mudd et al., 1997). Heath and Taylor (1997) cautioned there is little evidence that lipids are attacked by ozone in plants that have not been already severely injured by acute ozone exposure.

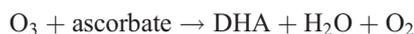
In the biological membrane, the double bonds of the fatty acids are spatially protected from ozone. In isolated membranes from yeast cells lipid modifications can be initiated by low ozone doses and develop proportionally to the treatment dose. However, in intact yeast cells even the most ozone-sensitive sterols and nitrogen-containing phospholipids did not undergo oxidative destruction at doses up to 6.0 μmol ozone mg protein^{-1} . It was suggested that these differences are related to both the function of antioxidative enzymes (catalase, superoxide dismutase, peroxidase) and the difference between the structural states (i.e., stability and acces-

sibility to oxidation) of lipids in the isolated membranes and the intact cells (Matus et al., 1999).

Reactions with lipids are not the primary target for the action of ozone, but rather proteins. The susceptibility of membrane-bound proteins depends on whether an oxidizable amino acid residue is at the active sites of the enzyme. Results from experiments with human RBC membranes suggest that both proteins and unsaturated lipids can undergo ozonation in RBC membranes, the lung and the plasma membrane of cells (Uppu et al., 1995). However, ozone is so reactive that it cannot penetrate far into the air–tissue boundary before it reacts (Pryor, 1994). This suggests that the identification of ozone reactions occurring in the intercellular fluid is critically important if we are to understand the mechanisms underlying the phytotoxicity of ozone. In addition, ozone interactions at the plasma membrane could change the ionic balances within the cell and affect regulation of intracellular calcium levels, for example, which would initiate diverse responses in the cell, possibly leading to metabolic imbalances (Heath, 1999).

3.1.2. The role of antioxidants

In concert with reactions between ozone and the symplast, reactions occur with metabolites, notably ascorbate, in the intercellular fluid. Apoplastic ascorbate has been proposed to constitute a first line of defense against ozone (Sharma and Davis, 1997). A model developed by Chameides (1989) identified extracellular ascorbic acid as a critical factor in the detoxification of ozone in the cell wall, shielding plasma membranes from oxidative damage and preventing ozone injury. In this model, ozone would be effectively scavenged through ozonolysis of ascorbate. Additional protection by ascorbate has been suggested through a reaction that yields dehydroascorbate (DHA) (Fig. 3), water and oxygen with a stoichiometry of 1:1 (Polle et al., 1995).



However, uncertainties remain regarding the stoichiometry and formation of intermediates during this reaction (Plöchl et al., 2000). Moreover, the relative importance of ascorbate in scavenging ozone in comparison with other understudied components of the extracellular fluid is unknown. Recent studies indicate that these additional scavenging metabolites almost certainly contribute to the protection against ozone afforded by the aqueous milieu of the cell wall (Turcsanyl et al., 2000; Maddison et al., 2002; Moldau and Bichele, 2002).

Ascorbic acid is synthesized inside the cell and is then transported into the extracellular space most likely by specific carriers presumed to exist in the plasma membrane (Horemans et al., 2000). Apoplast ascorbate content and redox status are reduced by ozone treatment (Castillo and Greppin, 1988; Luwe and Heber, 1995; Burkey, 1999), suggesting that extracellular ascorbic acid is involved in ozone detoxification. However, Kollist et al. (2000) have

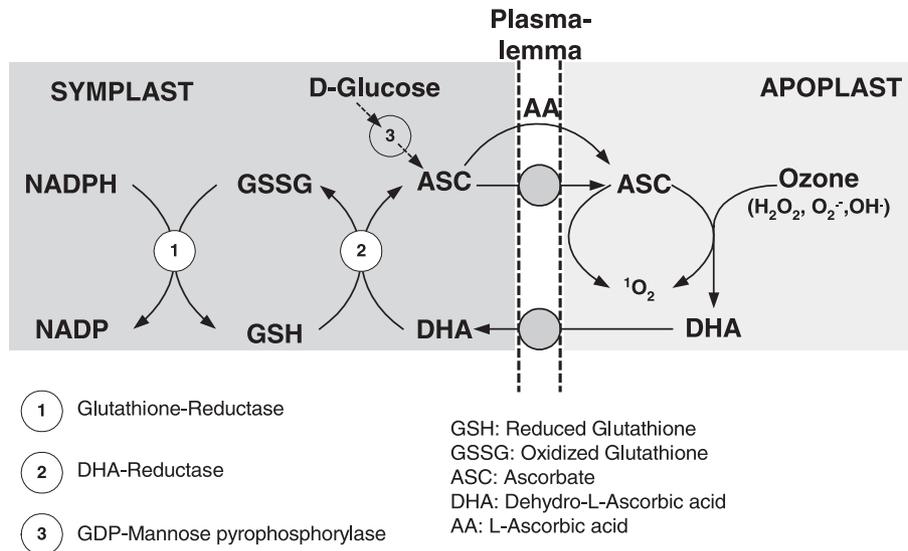


Fig. 3. Possible mechanism of detoxification. Ozone and its reactive derivatives react with ascorbate (ASC) in the leaf apoplast. ASC is supplied from the symplast via diffusion, facilitated by a transporter in the plasmalemma, and/or by diffusion of the neutral species (AA). Oxidized ASC in the form of DHA is returned to the symplast and reduced by DHA Reductase, utilizing GSH, or hydrolyzed to 2,3-diketo-L-gulonic acid. GSSG is in turn reduced by GSSG reductase which uses NADPH as reductant (redrawn with changes after Barnes et al., 2002).

calculated the possibility that only a part of the ozone flux entering the mesophyll cell surface is detoxified in the direct reactions with apoplastic ascorbate. It is probable that cell wall thickness and ascorbate concentration would affect detoxification efficacy and would vary among species.

It is not clear whether ascorbic acid reacts directly with ozone or indirectly as a substrate in antioxidant reactions, or both (Jacob and Heber, 1998). That ascorbate in the apoplast is highly reactive with ozone was indicated by measuring the chemiluminescence emission by singlet oxygen from the reaction of ozone with ascorbate in intercellular fluid extracted from *Sedum album* leaves (Kanofsky and Sima, 1995). The intensity of the singlet oxygen emission was decreased by 80% when ascorbate oxidase was added to the intercellular fluid. This suggests that ascorbic acid functions as a sacrificial antioxidant (Pryor, 1994; Kanofsky and Sima, 1995). In addition, the importance of ascorbic acid was demonstrated in the *VTC1* mutant of *Arabidopsis*, where reduced ascorbate content was associated with increased ozone sensitivity (Conklin et al., 1996). Recent studies have found higher levels of apoplastic ascorbic acid in both ozone-tolerant snap beans and *Plantago major* L. seedlings compared with sensitive lines. These results support the hypothesis that extracellular ascorbate contributes to tolerance (Zheng et al., 2000; Burkey et al., 2000; Burkey and Eason, 2002), but this relationship is not always observed (Guzy and Heath, 1993). Despite the controversial nature of results with respect to the involvement of ascorbate in ozone tolerance, interest in the manipulation of ozone resistance through the level of ascorbate in the apoplast remains high (Barnes et al., 2002). Targets may be enzymes involved in the synthesis of ascorbate or ascorbate oxidase.

It has been suggested that differential sensitivity to ozone exhibited by different varieties within a plant species is dependent on the relative amounts of ascorbate, polyamines, glutathione and the activities of enzymes associated with the ascorbate pathway and other antioxidant enzymes (Sharma and Davis, 1997). However, at this stage, it seems that screening for ozone tolerance based on antioxidant content may not be a reliable approach until we have a greater understanding of the key factors predicting tolerance to ozone responses at the molecular level.

A number of defense genes induced by pathogens are also induced by ozone (Sandermann, 1996, 1998). Most of these genes are not stress-specific and a number of stresses, including pathogens and ozone, are able to induce the same patterns of gene expression (Sävenstrand et al., 2000). This suggests that ozone acts as an abiotic elicitor of responses that are characteristic of hypersensitive response (HR) and results in systemic acquired resistance (Rao and Davis, 2001; Sharma and Davis, 1997). It is possible that some phenotypic effects of ozone are due to inappropriate expression of defense pathways. Recent studies suggest that cross-talk between salicylic acid (SA)-, jasmonic acid (JA)-, and ethylene-dependent signaling pathways regulates plant responses to a variety of stress factors (Pryor, 1994; Rao et al., 2000).

Programmed cell death (PCD), which leads to the formation of lesions typical of HR, may be activated by ozone through various pathways. Activated oxygen species (AOS) are suggested to be an important regulator of defense-gene expression and PCD during HR (Sharma and Davis, 1997). Ozone may serve as an inducer of the oxidative burst that appears to be a primary signalling step during the recognition of avirulent pathogens. Ozone exposure stimulated an oxidative burst in leaves of ozone-sensitive tobacco, tomato,

Arabidopsis, and several native European plants (*Malva sylvestris* L. and *Rumex* sp.) (Wohlgenuth et al., 2002). These ozone-derived AOS are similar to those induced by incompatible plant pathogens and initiate an array of signaling cascades (Rao and Davis, 2001). These authors proposed that in sensitive plants, ozone-derived AOS alter ion fluxes, activate phospholipases, protein kinases, and indirectly activate GTPases, mitogen-activated protein (MAP) kinases, NADPH-oxidase, amine oxidases and peroxidases. It was suggested that ozone-derived AOS and their effects on several enzymes increased production of AOS, nitric oxide (NO), SA, lipid hydroperoxides and ethylene, which are all known inducers of cell death (Rao and Davis, 2001). In animal studies, the aldehyde HNE induced PCD in murine alveolar macrophages and murine lung cells (Kirichenko et al., 1996). Formation of HNE adducts with proteins that may be associated with PCD and necrosis reflects the relative pathogenic importance of HNE as a secondary toxic messenger for acute ozone injury.

The accumulation of damage from ozone-derived AOS in addition to AOS-induced genes, whose products mediate senescence and degradation, are likely responsible for the accelerated senescence often observed following ozone exposure (Pell et al., 1997). Many of the predominant symptoms of ozone injury resemble senescence, such as chloroplast degeneration, protein loss, ethylene emission, decreased photosynthesis, chlorosis, necrotic lesions, and leaf abscission. One of the hallmarks of ozone-induced senescence is a decrease in Rubisco content along with decreased mRNA transcript levels for Rubisco and CAB (Pell et al., 1997). A number of genes show increased expression during senescence and are referred to as senescence-up-regulated genes or SAGs. Miller et al. (1999) found that eight SAGs were expressed in *Arabidopsis* plants following ozone treatment. Ozone treatment thus induced the early expression of many molecular markers of senescence.

In general, we now have a more comprehensive view of how ozone directly and indirectly affects plant metabolism, especially with regard to elicited antioxidant defense mechanisms. Clearly, a much better understanding of ozone chemistry in the apoplast is needed. Experiments indicate that protection from ozone by ascorbate is not complete, and that the potential roles of conjugated polyamines, phenolics and other anti-oxidative enzymes need to be examined (Sharma and Davis, 1997; Plöchl et al., 2000; Zheng et al., 2000; Maddison et al., 2002; Moldau and Bichele, 2002). Dissecting the interacting SA, JA and ethylene signal transduction pathways involved in plant defense responses to pathogens, ozone and other environmental factors is crucial to understanding plant responses to stress (Rao et al., 2000; Koch et al., 2000).

In addition, injury and SAG induction from ozone-derived AOS deserves attention, especially with regard to potential effects on protein trafficking and oxidative stress-sensitive steps involved in membrane renewal (D.J. Morre, personal communication).

3.2. Processes at the whole-plant level

Ozone impacts at the whole-plant level have received considerable attention, both in crops and native vegetation (Davison and Barnes, 1998). However, the current mechanistic understanding remains limited. As a consequence, good mechanistic models are lacking. While in the past, emphasis was placed on aspects directly related to productivity, such as photosynthesis, current research aims to elucidate mechanisms of ozone impacts on biomass partitioning, resource-use efficiency, and plant quality, all of which are strongly inter-linked. Importantly, in the context of future climate changes, these aspects will remain highly relevant. Additionally, improved basic understanding may help to tailor new crop varieties that are not only more tolerant to ozone, but also better adapted to projected future conditions (i.e., higher temperatures and reduced soil moisture) during the growing season. As an example, it may be possible to select for improved water-use efficiency by selecting for greater stomatal resistance, since increasing stomatal resistance in most plants decreases the rate of transpiration more than the rate of photosynthesis. This procedure could potentially also select for higher ozone tolerance due to increased ratio of photosynthesis to the absorbed ozone dose (CEL), a possibility that needs to be tested.

3.2.1. Carbon partitioning

Understanding allocation strategies for photosynthates is essential for the prediction of long-term responses of whole plants and agro-ecosystems to ozone. According to 'optimal partitioning models', adjustments in biomass allocation between above and below ground structures, in response to environmental stresses, may serve to balance resource acquisition and to maximize growth. However, these adjustments are highly variable, and often small or absent. Ozone stress may induce adjustments via the reduction of leaf carbon assimilation (i.e., by changing the source strength) (Minchin et al., 1993). This reduction in source strength could reduce the availability of soluble sugars, in source leaves, for export (Andersen et al., 1991). As observed in long-term ozone exposure studies with various crop and natural species, this effect is associated with shifts in the allometric coefficient due to a change in the growth rates of shoots relative to roots (Grantz and Farrar, 2000). Within the shoot, ozone reduces the harvest index in cereals by decreasing photosynthate allocation to grain (Fuhrer et al., 1989; McKee et al., 1997a). Alternatively, ozone effects on partitioning may be independent of effects on source strength. Recent work by Grantz and Farrar (1999, 2000) and Grantz and Yang (2000) with Pima cotton (*Gossypium barbadense* L.) suggests that the effects of ozone on carbon transport may dominate the effects of the pollutant on carbon assimilation.

Reductions in productivity were found to be related to increased non-structural carbohydrate contents of source leaves and inhibited carbon export to sinks such as roots,

reproductive structures and shoot meristems. The reduced flux of carbon could not be explained by reduced source strength because it differed from effects of other treatments reducing biomass and leaf area similarly to ozone.

This points to a direct effect of ozone on phloem loading that results from ozone-induced oxidation of a sensitive protein involved in phloem loading, such as the sucrose translocator. Seen as the likely cause, Grantz and Farrar (2000) further suggested that this could lead to apparent preferential transport to adjacent sinks (e.g., stems) at the expense of distant sinks (e.g., roots). In contrast, no effect of ozone was found on the proton and sucrose transport at the plasma membrane of *Ricinus communis* (Russell et al., 1999). These results stress the need for a critical evaluation of the effects of ozone on phloem loading, particularly under long-term exposure conditions, and in different species and cultivars. The use of transgenic plants as tools to test the various hypotheses could be helpful. Also, an attempt should be made to link the changes in carbon translocation to the effective ozone dose resulting from non-scavenged oxygen species after ozone uptake.

There are a number of expected consequences of reduced carbon flux to below ground structures: (1) changes in hydraulic function of roots leading to altered whole-plant water relations; (2) reductions in carbon transfer from the plant to the soil; (3) altered soil nutrient uptake by the roots. These consequences are significant in the light of projected future climatic conditions that may be associated with higher evaporative demands and drier soils during the growing season. Reduced water and soil nutrient uptake antagonize efforts to increase the nutrient- and water-use efficiencies of crops and are thus in conflict with the interest to increase sustainable land management. Furthermore, given the current interest in carbon sequestration in agricultural soils, it is important to know that a reduction in carbon input to soils, due to reduced root growth, will diminish the agricultural potential for greenhouse gas mitigation. The latter aspect becomes even more important in perennial crops and forages.

3.2.2. Interactions

In the real world, effects of ozone on plants rarely occur in the absence of affects of other environmental influences and

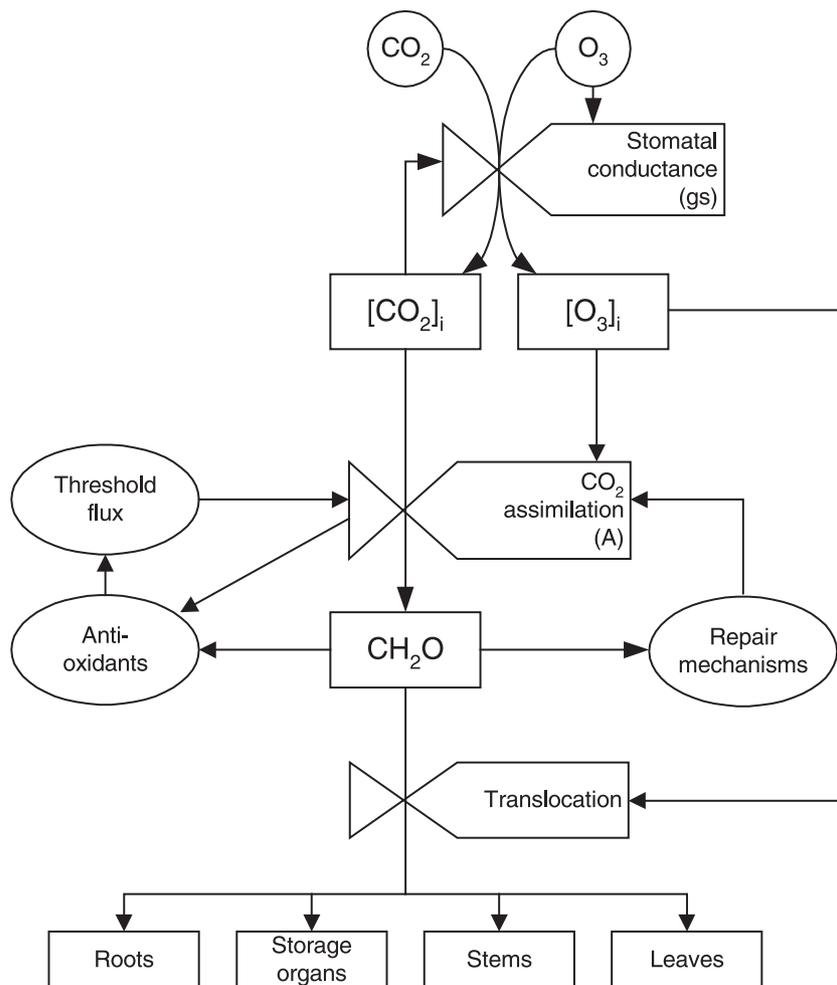


Fig. 4. Conceptual model for the interaction of CO₂ and ozone on CO₂ assimilation and photosynthate (CH₂O) allocation, involving links to detoxification and repair.

limiting factors. Thus, efforts to increase plant performance under future conditions by breeding or biotechnological progress will depend on the understanding of these interactions. Many of them are poorly understood and deserve more basic research attention. An important underlying principle may be that the extra factors affect either ozone uptake or the mechanisms of detoxification and/or repair necessary to cope with ozone stress, as described below for the ozone–CO₂ interaction. However, an overall conceptual model integrating the influence of various factors, including UV-B radiation, temperature, CO₂ concentration, other gaseous pollutants, or particulate matter is lacking.

A focus of ongoing research is the interaction between ozone and atmospheric CO₂ (Fig. 4). The presence of elevated ozone in the atmosphere affects the magnitude of the yield-enhancement by elevated CO₂. Experiments with wheat, soybean, and cotton demonstrated that the positive effect of elevated CO₂ on yield was larger in an atmosphere containing elevated levels of ozone (Heagle et al., 1998, 1999, 2000). However, potato tuber yield was increased by elevated CO₂ to a similar extent at low and high ozone concentrations (Donnelly et al., 2001). Considerable evidence suggests that ozone uptake is decreased by elevated CO₂. According to current understanding, plant biomass production is largely protected from ozone effects in a CO₂-rich atmosphere due to ozone exclusion, rather than increased detoxification capacity (Fiscus et al., 1997; McKee et al., 1997a). However, the two processes act in concert; in plants treated with elevated CO₂ ozone uptake is reduced and the availability of carbon for detoxification is increased (Heagle et al., 1999).

The protection from ozone damage, provided by elevated CO₂, appears to be due to the protection of the photosynthetic system. It was suggested that less protection is expected in terms of processes not directly related to photosynthesis, such as reproduction (McKee et al., 1997b, 2000). This would imply that, in some cases, protection by elevated CO₂ may not be as efficient in the case of reproductive growth, as it is in the case of vegetative growth. Hence, an overall scheme to describe ozone effects on biomass allocation, which accounts for interactions with other relevant environmental factors, should consider interactive effects on source/sink relationships, as well as the direct effects of ozone on structural elements involved in carbon translocation, and direct effects of ozone on reproduction.

It is known that the effects of ozone on crop physiological processes may also be influenced by soil moisture availability. Reduced soil moisture may limit ozone access by reducing stomatal conductance, and hence protect cellular components from oxidant attack. However, recent findings suggest that, in some species, soil moisture stress may reduce rather than increase ozone tolerance (Bungener et al., 1999). This may operate via negative effects of water stress on cellular defense systems. Further, important interactions may occur between the effects of ozone and salinity. Under the assumption that irrigation expands with projected climate

changes, salinization of soils may also become a serious concern. Last but not least, the interactions between the effect of ozone and increasing temperatures becomes important as global temperatures rise. Current knowledge concerning temperature modulation of ozone responses is very limited, and additional research with properly designed experimental equipment is necessary.

4. Issues related to long-term effects of ozone

4.1. Yield and quality

Long-term effects of ozone on annual crops result from the cumulative impact of ozone taken up over the course of a single growing season. From an agronomic point of view, yield and nutritional quality are of the utmost importance—with the former aspect being more extensively studied in the past than the latter. Hence, there may be somewhat less incentive to carry out additional yield loss studies in the US and in Europe, whereas studies into nutritional aspects may be more attractive. Changes in quality traits have been studied in a limited number of crops. In wheat, the most important effects of ozone include increases in grain protein concentration (Fuhrer et al., 1990; Pleijel et al., 1999). In a 2-year experiment with *Gossypium hirsutum* L., lint from plants treated with ozone in open-top chambers had lower micronaire, elongation, uniformity and yellowness, and increased fiber length and brightness (Heagle et al., 1999). In oilseed rape, ozone has been found to reduce the oil, protein, and carbohydrate contents of the seed (Bosac et al., 1998). In *Glycine max* (L.) Merr., seeds from plants treated with ozone showed small changes in percentage oil, no changes in protein, minor suppression of oleic acid and small increases in linoleic acid concentration (Heagle et al., 1998). None of the latter effects of ozone on seed quality, however, appeared large enough to be of economic importance. In potato tubers, ozone was found to have positive effects on quality by decreasing the content of reducing sugars and increasing the vitamin C content (Vorne et al., in press).

In many developed countries, domestic agricultural production levels are sufficiently high that changing consumer preferences may become more important in driving research in the coming years. Improvement of the nutritional value of crops is an issue for the food industries, and studies on the possible impacts of ozone on nutritional aspects need to be considered. However, it is difficult to project particular crops for future ozone research. Candidates may be food crops that are important for a well-balanced vegetarian diet. In countries where meat consumption is in decline, increased production of protein through cultivation of soybean may be required. Also, screening efforts to select ozone-tolerant crops and cultivars will remain important to secure sufficient food supply in the developing world. An increase in production could be achieved by intensification (i.e., by increasing the yield per hectare), or extensification (i.e., by

expanding the area of cultivated land). In many countries the possibility for expanding the area available for production is limited. Therefore, there will be a continuous need to increase crop productivity on land currently available (Gregory and Ingram, 2000), and hence losses in productivity due to ozone may have severe consequences. Studies with local varieties of wheat and rice crops in Pakistan have revealed potentially large effects of ozone on crop yields (Maggs et al., 1995; Wahid et al., 1995; Maggs and Ashmore, 1998). Thus, it is important to expand the studies of ozone effects on food crops, such as wheat and rice, in parts of South and Southeast Asia. In these areas, it may be possible to test newly developed varieties for ozone tolerance, and/or develop new ozone-tolerant cultivars through targeted breeding from ozone-tolerant germplasm. Projections of the demand for different crop types to 2007 have been prepared by the US Department of Agriculture (USDA, 1998). These projections indicate, for instance, that in China vegetable production may double between 1991 and 2010 (Verburg et al., 2000). Such projections could indicate priority crops for future studies. However, the production of non-food crops may become increasingly important (e.g., energy crops), and potential effects of ozone on these crops is lacking.

4.2. Effects on grasslands

In the case of perennial crops and grasslands (pastures and rangelands), relevant long-term effects of ozone may develop over several years. An important aspect is the potential for change in forage quality, an issue that has received relatively little attention in the past. Forage quality may be changed because of ozone effects on leaf chemistry. This could be a direct effect on secondary metabolism, or a change in plant development. Long-term ozone exposure can lead to increased levels of phenolic acids, flavonoids and related compounds (Booker and Miller, 1998) that may negatively affect ruminant microorganisms and enzyme systems. One of the most common effects of ozone is to promote leaf senescence. Thus, in pastures or other types of grasslands exposed to ozone the fraction of senescing tissue may be increased. Because of increased lignification, and a decreased leaf/stem ratio, forage digestibility would decline. Quantification of effects, however, is lacking, and the consequences for animal nutrition need to be studied in more detail. In grass-clover forage, white clover (*Trifolium repens* L.) leaf in vitro dry matter disappearance (IVDMD) and N were decreased, and neutral detergent fiber (NDF) increased by ambient ozone (50 nl l^{-1}) compared with charcoal-filtered air (Burns et al., 1997). A major deficiency common to earlier studies of ozone effects on forage quality is their use of outdated procedures to analyze and fractionate plant carbohydrate into crude fiber and non-structural carbohydrates (R.B. Muntifering, personal communication). Using an improved methodology, Muntifering et al. (2000) have reported decreased dry matter yield and quality

of ozone-exposed bahia grass of sufficient magnitude to have nutritional implications for its utilization by ruminant animals.

Changes in forage quality may result from shifts in species composition. Differential ozone sensitivities between grasses and legumes have been found to cause a shift in the grass/legume ratio in favor of grasses, and, hence, in protein concentration and other quality traits relevant for animal nutrition (Rebbeck et al., 1988; Fuhrer, 1997). However, knowledge concerning effects of ozone on the floristic composition of permanent pastures and rangelands is limited. To some extent, this is due to limitations in past experiments; short-term experiments with sown mixtures exposed to ozone in open-top chambers may not realistically reflect the situation under ambient conditions, and the results may not be applicable to established permanent grassland systems used for grazing, silage or hay production. Furthermore, plant–plant interactions may respond to both ozone stress and soil moisture conditions (Nussbaum et al., 2000). Preliminary results (unpublished) are available from a study of long-term effects of elevated ozone on forage species composition and quality, using an open-air fumigation system in Switzerland. The results show a much weaker responses, in terms of species composition, after several years of exposure than observed in earlier open-top chamber experiments with sown mixtures (Fuhrer et al., 1994). The reasons for the lack of a rapid response at the community level are unclear. It could be that plants with established root systems may be more tolerant to ozone episodes than plants in communities in the establishment phase. Experimental treatments in open-top fumigation chambers may exacerbate the effect of ozone because of different microclimatic conditions as compared to those in ambient air (Nussbaum and Fuhrer, 2000). Also, the effect of ozone on perennial grassland systems depends not only on the effects during ozone episodes, but also on the response of the plant mixtures to reduced ozone levels between the episodes. Finally, little is known about the indirect effects of ozone on seed output and seed viability via changes in carbon assimilation and allocation, and virtually nothing is known about the direct effects on reproductive processes. Existing experimental data do not allow the assessment of these various possibilities and, hence, the question of long-term effects on forage quality and biodiversity remains open. Long-term experimentation with properly designed and innovative exposure facilities will be essential in these studies.

4.3. Nutrient cycling and carbon sequestration

Ozone has been found consistently to increase the nitrogen concentration in wheat grain (Pleijel et al., 1999), whereas little is known about the effects on residues. Effects of ozone on the concentration of secondary metabolites, lignification, and/or the C/N ratio of above- and below-ground plant parts may change the biodegradability of litter and thus the rate of decomposition and the nutrient and

carbon turnover in the soil. Data from a study with blackberry and broomsedge bluestem indicated that ozone affects substrate quality and soil microbial activity, resulting in reduced rates of litter decomposition (Kim et al., 1998). This aspect must be considered in the context of reduced carbon transfer to roots and the soil. The effects of ozone on plant and residue quality may translate into long-lasting changes in soil chemical and microbiological properties. Relevant in the global change context is the effect on carbon pools in grassland soils. Improved estimates of potential soil carbon sequestration under different management regimes and climate scenarios need to take into account the effects of ozone.

Another aspect of the plant–soil relationship concerns mycorrhizae. Most land plants form mycorrhizae, so the role of mycorrhizae in mediating plant responses to atmospheric change may be an important consideration in predicting effects of ozone on plants in managed and natural ecosystems (Shafer and Schoeneberger, 1991). However, their role in plant and community responses to ozone has received little attention, and thus few reliable quantitative generalizations can be made. Ozone suppresses carbon allocation to roots (see above), and the dependency of the VAM endophyte on plant-supplied carbon renders the fungus indirectly subject to ozone-induced stress. Due to changes in root growth, changes in the mycorrhizal status of the plant may be impacted early in the plant–ozone interaction and may actually condition other plant responses to the stress, or ozone may impair the mycorrhizae-induced protection from root pathogens (Shafer and Schoeneberger, 1994). Recent experiments with wheat suggest that exposure to ozone causes an increase in ¹⁴C-labeled photosynthate released into the soil solution surrounding the root tissue (McCrary and Andersen, 2000). This root exudation is the primary carbon source for most of the microbial activity in the rhizosphere, and a small change would be expected to alter the rhizosphere microbial activity. Clearly, additional quantitative descrip-

tions of relations among environmental stresses, plant physiology, mycorrhizal infection, and mycorrhizal function will be needed before the importance of mycorrhizae in ozone-related changes in the ecosystem can be assessed.

5. Conclusions

Future research on ozone effects on agricultural crops is challenged by the need for dynamic ozone risk analyses on regional scales. Other challenges include the development of ozone tolerant crops and the mitigation of ozone for sustainable agro-ecosystem management (Fig. 5).

Specifically, the following aspects should be considered in future research programs:

- Modelling of ozone transfer and specifically stomatal ozone uptake under variable environmental conditions, using robust and well-validated dynamic models that can be linked to large-scale photochemical models.
- Processes involved in the initial reactions of ozone with extracellular and cellular components, after entry through the stomata, must be understood. It is not until the role of apoplast constituents and the detoxification of ozone or the products of ozonolysis are better characterized that mechanistic models can be developed. These can then be linked to ozone uptake models and targets for crop improvements using modern techniques can be identified.
- Scaling the effects at the level of individual cells to the whole plant level is critical. This requires a better understanding of the effects of ozone on carbon transport within the plant. A key process linking cellular with whole plant effects may be phloem loading. Progress in this area will be crucial for modelling long-term effects at the system level. Long-term predictive models are required as the basis for the development of mitigation and adaptation strategies in agro-ecosystem management.

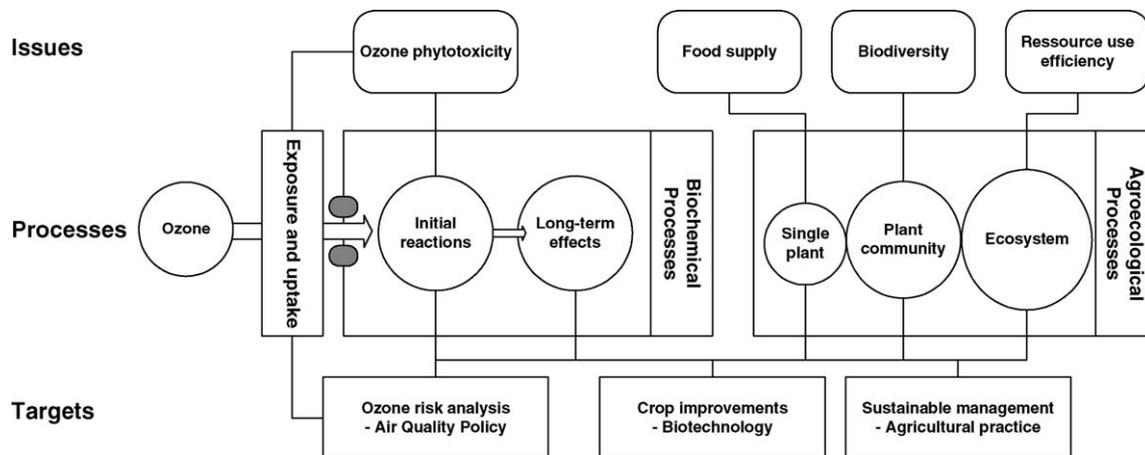


Fig. 5. Summary of the different key agricultural issues in relation to ozone pollution, relevant processes that need research attention, and target areas for applications.

- The effects of long-term ozone exposures, on individual plants, is important to understand community and whole-system level processes. Important aspects identified are (1) changes in the quality of crops used for human nutrition or animal feed, (2) nutrient cycling and nitrogen/carbon balances in agro-ecosystems, (3) changes in biodiversity of pastures and rangelands, and agricultural landscapes. The latter aspect becomes increasingly important in areas where serious and costly attempts are being made to increase biodiversity in agricultural landscapes.
- Interactions between ozone and other environmental factors that are either directly manipulated by man, such as fertilization, crop management and others, or indirectly changed as part of global change, including elevated atmospheric CO₂, particulate matter, other pollutants such as nitrogen oxides, UV-B radiation, climate and associated soil moisture conditions.

In order to be effective in a policy, or in a technological context, the results from future research should be funnelled into an appropriate knowledge transfer scheme to gain added value. This requires data synthesis, up-scaling, and spatial aggregation. The example of the UNECE Convention on Long-Range Trans-boundary Air Pollution demonstrates how policy decisions may require the use of integrated assessment modelling. Because such models depend on quantitative exposure–response information, it is necessary to link the effects at each level of organization quantitatively to an effective ozone dose, which is the balance between the rate of ozone uptake by the foliage and the rate of detoxification.

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